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(71) Applicant: 000113470
Pola Chemical Industrial Company, Ltd.
6-48 Yayoi-cho, Shizuoka-shi, Shizuoka-ken

(72) Inventor: Akio Fujii
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Yasuji Ogo
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Kenkichi Matsubara
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

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(54) Title of the Invention: A Topical Skin Agent

(57) [Abstract]

[Structure] A melanin production inhibitor that is comprised of essence of seeds of plants of the family Curcurbitaceae and a topical skin agent that contains them.

[Effect] The melanin production inhibitor of this invention is of high safety and superior melanin production inhibiting action. Topical skin agents in which it is compounded have superior beautifying-whitening action.

[Claims]

[Claim 1] A melanin production inhibitor that is comprised of essence of seeds of plants of the family Curcurbitaceae.

[Claim 2] A melanin production inhibitor as described in Claim 1 in which the plant of the family Curcurbitaceae is Benincasa hispida Thummb Cogn..

[Claim 3] A topical skin agent that contains the melanin production inhibitor as described in Claims 1 and 2.

[Claim 4] A topical skin agent as described in Claim 3 in which the content of melanin production inhibitor as described in Claims 1 and 2 is 0.0001 to 10 weight %.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to a melanin production inhibitor and to a topical skin agent that contains it. In greater detail, [this invention relates to] melanin production inhibitors that are of essence of seeds of plants of the family Curcurbitaceae and a topical skin agent that contains them.

[0002]

[Prior art] It is the desire of many women to have white, beautiful skin. Skin that was white during infancy gradually undergoes pigment deposition and loss of beauty over the years or due to the effects of ultraviolet rays. Pigment deposition is thought to occur when melanin is produced as tyrosine is changed to dopa and dopa is changed to dopa quinone by the enzyme tyrosinase, which is biosynthesized in pigment cells and via intermediates such as 5,6-dihydroxyindole.

[0003] From this standpoint, sulfur containing compounds such as glutathione and hydroquinoine derivatives, which have an inhibiting action of tyrosinase activity and hydrogen peroxide solutions, ascorbic acid and derivatives thereof which bleach the melanin that is produced have been developed as substances for inhibiting pigment deposition. However, these substances present problems in terms of safety, decomposition, coloration and generation of unpleasant odors. In addition, there are also not a few problems of safety such as formation of irreversible white spots and occurrence of rashes. Consequently, it is no exaggeration to say that melanin production inhibitors fit for practical use have not yet been developed.

[0004] On the other hand, it is known that seeds of plants of the family Cucurbitaceae have a melanin production inhibiting action.

[0005]

[Problems the invention is intended to solve] This invention was developed in the light of these circumstances. Consequently, it has the objective of providing melanin production inhibitors of superior safety and melanin production inhibition and cosmetic materials that contain them.

[0006]

[Means for solving the problems] On the basis of the viewpoint described above, the inventors collected raw drugs that were thought to be of superior safety and concerning which there were records of use for many years and they conducted repeated screenings with melanin production inhibiting action as the index and perfected this invention by discovering that winter melon seeds, which are seeds of Benincasa hispida Thummb Cogn. of the family Curcurbitaceae have a marked melanin production inhibiting action.

[0007] Specifically, this invention relates to melanin production inhibitors comprised of essence of seeds of plants of the family Curcurbitaceae.

[0008] This invention further relates to topical skin agents that contain melanin production inhibitors comprised of essence of seeds of plants of the family Curcurbitaceae.

[0009] We shall now present a detailed description of this invention. Seeds of plants of the family Curcurbitaceae that are used as the melanin production inhibitors in this invention are widely used for various purposes as a Chinese traditional medicinal drug. For example, trichosanthis seed, which are seeds of Korean crow gourd [Translator's Note: Literally translated from the Japanese. We were not able to reference this material in the various specialized glossaries available to us.], are used for moisturizing the lung and transforming phlegm and for ulcers. Toka seeds, which are seeds of Benincasa hispida Thummb Cogn. are used for clearing the lungs, transforming phlegm, disinhibiting dampness and expelling pus [Translator's Note: These are terms used in traditional Chinese medicine.]. Of the seeds of plants of the family Curcurbitaceae that are used in this invention, the most desirable are tokashi, which are seeds of Benincasa hispida Thummb Cogn.. This is because Benincasa seeds are of superior melanin production inhibiting action and safety.

[0010] The term essence of seeds of plants of the family Curcurbitaceae of this invention refers to seeds of plants of the family Curcurbitaceae in unaltered state, to seeds that have been dried and finely pulverized, to extracts obtained by subjecting the seeds to extraction with a polar solvent and removing the solvent and to fractions obtained by fractionating extracts by column chromatography or fluid separation extraction.

[0011] There are methods for obtaining extracts from seeds of plants of the family Curcurbitaceae. They can be performed by ordinary extraction methods. Before the extraction procedure, it is desirable to perform pre-treatments such as drying and pulverizing the seeds in advance. In these cases, solvent is added to the seeds or pretreated seeds in amounts of 1 to 100 times their volume. They are immersed for several hours as the temperature is being raised or for several days if they are at room temperature and the insoluble matter is removed by filtration, after which they may be dried under reduced pressure and the solvent removed. Solvents of high polarity are particularly desirable. For example, there can be one or two or more solvents selected from water, methanol, ethanol, propanol, butanol, acetone, diethyl ether, chloroform, methylene chloride and dichloroethane.

[0012] The extract that is obtained in this way may be compounded in an unaltered state with the topical skin agent and it may also be compounded after fractionation and purification by column chromatography or liquid-liquid extraction. As specific examples of fractionation and purification we can cite silica gel chromatography using a mixed solution of methanol and diethyl ether or a mixed solution with chloroform as the elution solvent, ODS column chromatography in which a methanol aqueous solution or an acetone aqueous solution as the elution solvent and liquid-liquid extraction with butanol-water, diethyl ether-water, ethyl acetate-water and hexane-water.

[0013] The essence that has been obtained in this way can be made into a topical skin agent preparation with various optional components in accordance with ordinary methods. There are no particular limitations on the form of the preparation as long as it is a form that is ordinarily used such as a lotion, a cream, an ointment an emulsion or a pack. Optional components can include polyvalent alcohols, humectants, thickeners, hydrocarbons, esters, alcohols, higher fatty acids, surfactants, powdered components, colorants, fragrances, antioxidants, ultraviolet ray absorbents and antiinflammatory agents. In addition, other melanin production inhibitors such as sulfur-containing compounds, ascorbic acid derivatives and hydroquinone derivatives may also be compounded.

[0014] The content of melanin production inhibitor of this invention in the topical skin agent should be 0.0001 to 10 weight %. When it is less than 0.0001 weight %, melanin production inhibiting activity cannot be anticipated. When it exceeds 10 weight %, the effect is already at its maximum and the increased amount is not economical. It is preferable that the content of melanin production inhibitor of this invention in the topical skin agent be 0.1 to 10 weight %, at which level the melanin production inhibiting action is at a maximum.

[0015]

[Examples] We shall now describe this invention in greater detail by presenting examples. However, it goes without saying that this invention is not limited by these examples.

[0016] Example 1

Example of Manufacture

1 liter of a 50% aqueous solution of methanol was added to seeds of Benincasa hispida Thummb Cogn., heating and reflux were performed for 3 hours and 7.5 g of melanin production inhibitor 1 was obtained as a pale yellow amorphous substance.

[0017] Example 2

Example of Manufacture

7.5 g of melanin production inhibitor 1 as described above was dispersed in 300 ml of water, 300 ml of normal hexane was added and the mixture was thoroughly agitated, after which solution separation was performed, the normal hexane layer was removed and dried under reduced pressure, with 170 mg of melanin production inhibitor 2 being obtained as a pale yellow amorphous substance. 300 ml of ethyl acetate was added to the remaining aqueous layer and the mixture was thoroughly agitated, after which the ethyl acetate layer was collected and dried under reduced pressure, with 360 mg of melanin production inhibitor 3 being obtained as a pale yellow amorphous substance. 300 ml of normal butanol was added to the remaining aqueous layer and the mixture was thoroughly agitated, after which the butanol layer was collected and dried under reduced pressure, with 850 mg of melanin production inhibitor 4 being obtained as a pale yellow amorphous substance. The remaining aqueous layer was dried under reduced pressure and 5960 mg of melanin production inhibitor 5 was obtained as a pale yellow amorphous substance.

[0018] Example 3

Melanin production inhibiting action

A study was conducted of the melanin production inhibiting action of the melanin production inhibitors 1 to 5 obtained in Examples 1 and 2 using melanoma B-16 cells. Specifically, B-16 cells in the logarithmic growth period were treated with trypsin, a MEM culture medium suspension of 1.5×10^3 cells/ml containing 10% FBS (bovine fetal serum) was prepared and this suspension was poured in amounts of 10 ml each into individual culture bottles. They were cultured for 2 days in a CO_2 incubator (37°C, 5% CO_2). Various concentrations of melanin production inhibitor were added and culturing was continued for 2 days. The culture medium was discarded on the 6th day and the culture was washed with PBS (phosphate buffer physiological saline solution), after which trypsin treatment was performed and the cells were peeled off. Following this, the cells were collected by centrifugation and were observed visually, with evaluations being made for cytotoxicity and melanin production inhibitory action. The criteria for cytotoxicity were as follows. +: cytotoxicity was present; ±: evaluation of cytotoxicity was difficult; -: cytotoxicity was not present. The criteria for melanin production inhibitory action were as follows: +: melanin production inhibitory action was present; ±: very slight melanin production inhibitory action was found; -: melanin production inhibitory action was not present. The results are shown in Table 1. It was found that the melanin production inhibitors of this invention inhibited the production of melanin satisfactorily even at low concentrations.

[0019]

[Table 1]

Samples	Concentration (%)	Melanin production inhibition	Cytotoxicity
Melanin production inhibitor 1	0.1		
Melanin production inhibitor 2	0.004 0.002 0.0002	+ + ±	± - -
Melanin production inhibitor 3	0.01 0.005	+ ±	+ -
Melanin production inhibitor 4	0.02	±	-
Melanin production inhibitor 5	0.17	±	±

[0020] Example 4

Safety (local toxicity)

In order to ascertain the safety of the melanin production inhibitors of this invention, a local toxicity study was conducted by percutaneous injection using Hartley white guinea pigs (males, 350 g). Specifically, 1% ethanol solutions of melanin production inhibitors 1 to 5 were administered 5 times a day in doses of 0.05 ml each into 2 cm square sites that were made by shaving the backs of the guinea pigs. On the 6th day, the skin reaction was evaluated on the basis of the Japanese Patch Test Criteria (Japanese Dermatology Society); specifically. -: no reaction; ±: false positive reaction; +: positive reaction; ++: reaction accompanied by edema. The results were negative reactions (-) in all cases. Thus, it was found that the melanin production inhibitor of this invention exhibited excellent safety.

[0021] Example 5

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	83.1
Melanin production inhibitor 1	0.1

[0022] Example 6

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	82.2
Melanin production inhibitor 2	1

[0023] Example 7

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	73.2
Melanin production inhibitor 1	10

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

[0024] Example 8

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	83.1
Melanin production inhibitor 3	0.01

[0025] Example 9

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5
(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 2	0.03
	Water	56.4
	Propylene glycol	8
	Methylparaben	0.25

[0025] Example 10

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5

(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 1	0.0002
	Water	56.4298
	Propylene glycol	8
	Methylparaben	0.25

[0026] Example 11

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5
(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 5	0.3
	Water	56.13
	Propylene glycol	8
	Methylparaben	0.25

[0027] Example 12

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5

(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 1	0.003
	Water	56.427
	Propylene glycol	8
	Methylparaben	0.25

[0028] Example 13

Example of Compounding (Foundation)

A foundation was prepared in accordance with the formulation indicated below. Specifically, A was mixed by kneading, after which B was added and the materials were further mixed by kneading. The materials were heated to 80°C, after which C was dispersed and D, which had been dissolved by heating at 80°C was gradually added and emulsification was effected. The materials were stirred and cooled, with a foundation being obtained.

(A)	Propylene glycol	5
	Malvitol [phonetic]*	10
	Methylparaben	0.3
	Glycerol triisostearate	4
(B)	Liquid paraffin	5
	Butylparaben	0.1
(C)	Titanium oxide	9
	Yellow iron oxide	1.7
	Red iron oxide	1.2
	Talc	8.1
(D)	Water	55
	Melanin production inhibitor 5	0.6

[0029]

[Effect of the invention] The melanin production inhibitors of this invention exhibit excellent safety, and, moreover, their melanin production inhibiting activity is also high, for which reason they are very advantageous. Consequently, topical skin agents that contain them are also safe and have superior melanin production inhibiting action and are therefore very advantageous.

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(72) Inventor: Takeshi Hara
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Satoru Obata
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

*Translator's Note: Transliterated from the Japanese. As such, the spelling may differ from other transliterations.

(72) Inventor: Takuji Nakajima
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Yoshiyuki Miyata
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Yoshio Kitada
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

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(71)出願人　000113470
ポーラ化成工業株式会社
静岡県静岡市弥生町６番48号

(72)発明者　藤井　昭男
神奈川県横浜市神奈川区高島台27番地１
ポーラ化成工業株式会社横浜研究所内

(72)発明者　大郷　保治
神奈川県横浜市神奈川区高島台27番地１
ポーラ化成工業株式会社横浜研究所内

(72)発明者　松原　顕吉
神奈川県横浜市神奈川区高島台27番地１
ポーラ化成工業株式会社横浜研究所内

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(54)【発明の名称】　皮膚外用剤

(57)【要約】
【構成】　瓜科植物の種子のエッセンスからなるメラニン生成阻害剤及びそれを含有する皮膚外用剤。
【効果】　本発明のメラニン生成阻害剤は安全性が高い上、メラニン生成阻害作用に優れる。これを配合した皮膚外用剤は優れた美白作用を有する。

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【特許請求の範囲】

【請求項1】　瓜科植物の種子のエッセンスからなるメラニン生成阻害剤。

【請求項2】　瓜科植物が瓜科冬瓜である、請求項1記載のメラニン生成阻害剤。

【請求項3】　請求項1又は2記載のメラニン生成阻害剤を含有する皮膚外用剤。

【請求項4】　請求項1又は2記載のメラニン生成阻害剤の含有量が0．0001～10重量%である、請求項3記載の皮膚外用剤。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明はメラニン生成抑制剤及び皮膚外用剤に関する。更に詳しくは、瓜科植物の種子のエッセンスからなるメラニン生成抑制剤及びそれを含有する皮膚外用剤に関する。

【0002】

【従来の技術】白く美しい肌を持つことは多くの女性の願望であった。赤ん坊の時は白かった肌も、年を経るにしたがって、或いは、紫外線などの影響によって、次第に色素沈着がかさみその美しさを失ってしまう。このような色素沈着は、色素細胞中で生合成された酵素、チロシナーゼにより、チロシンからドーパ、ドーパからドーパキノンに変化し、5，6－ジヒドロキシインドール等の中間体を経てメラニンが生成され起こるものとされている。

【0003】かかる視点から、色素沈着を抑制させるものとして、チロシナーゼの活性阻害作用を有する、グルタチオン等の含硫化合物やヒドロキノン誘導体等が、又、生成したメラニンを漂白する、過酸化水素水やアスコルビン酸及びその誘導体が開発されてきたが、これらは、安定性上、分解や着色や悪臭の発生等の問題があるほか、不可逆的な白斑の形成やかぶれなどの安全性上の問題も少なくなかった。したがって、実使用に耐えるメラニン生成阻害剤はまだ開発されていないと言っても過言ではない。

【0004】一方、瓜科植物の種子がメラニン生成阻害作用を有している事は知られていなかった。

【0005】

【発明が解決しようとする課題】本発明は、かかる実情を鑑みて為されたものであり、従って、安全性及びメラニン産生抑制に優れたメラニン生成阻害剤及びそれを含有する化粧料を提供することを課題とする。

【0006】

【課題を解決するための手段】上記視点に立ち、本発明者らは、安全性に優れていると思われる、長年にわたる使用実績のある、生薬を集め、メラニン産生抑制作用を指標にスクリーニングを重ね、瓜科冬瓜の種子である冬瓜子が著しいメラニン生成阻害作用を有する事を見出し発明を完成させた。

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【0007】即ち、本発明は、瓜科植物の種子のエッセンスからなるメラニン生成阻害剤に関する。

【0008】又、本発明は、瓜科植物の種子のエッセンスからなるメラニン生成阻害剤を含有する皮膚外用剤に関する。

【0009】以下、本発明について詳細に述べる。本発明でメラニン生成阻害剤として用いる瓜科植物の種子は、漢方生薬としていろいろな目的で広く用いられていた。例えば、瓜科朝鮮烏瓜の種子である、カロシは潤肺化痰や潰腸の目的で、瓜科冬瓜の種子である冬瓜子は清肺、化痰、利湿、排膿等の目的で用いられてきた。本発明で用いる、これら瓜科植物の種子のうちで最も好ましいものは、瓜科冬瓜（Benincasa hispida Thummb Cogn.）の種子である冬瓜子である。これは、冬瓜子がメラニン生成阻害作用及び安全生に優れるからである。

【0010】本発明の瓜科植物の種子のエッセンスとは、これら瓜科植物の種子をそのまま、或いは乾燥して、細かく粉砕したもの、種子を極性溶媒で抽出し溶媒を除去した抽出物、抽出物をカラムクロマトグラフィーや分液抽出により分画した分画物を言う。

【0011】瓜科植物の種子より抽出物を得る方法であるが、これは通常の抽出方法にしたがって行えば良く、抽出操作に先だって、種子を予め乾燥したり、粉砕したりして前処理しておいても良い。これら、種子或いは種子の前処理物に溶媒を1～100倍量加え、加温下ならば数時間、室温下であれば数日浸漬し、濾過等により不溶物を除去した後、減圧乾固等して溶媒を除去すれば良い。用いる溶媒としては極性の高いものが好ましく、例えば、水、メタノール、エタノール、プロパノール、ブタノール、アセトン、ジエチルエーテル、クロロホルム、塩化メチレン、ジクロロエタン等から選ばれる1種又は2種以上が挙げられる。

【0012】斯くして得られた抽出物はそのまま皮膚外用剤に配合しても良いが、更にカラムクロマトグラフィーや液液抽出により分画精製した後配合しても良い。分画精製の具体的な例示としては、メタノールとジエチルエーテルとの混合溶液やクロロホルムとの混合溶液を溶出溶媒としたシリカゲルカラムクロマトグラフィー、メタノール水溶液やアセトニトリル水溶液を溶出溶媒としたODSカラムクロマトグラフィー、ブタノール－水、ジエチルエーテル－水、酢酸エチル－水、ヘキサン－水等の液液抽出が挙げられる。

【0013】斯くして得られたエッセンスは、各種任意成分と共に、通常の方法に従って皮膚外用剤へと剤形化できる。剤形としては、ローション、クリーム、軟膏、乳液、パック等通常のものであれば、特に限定はない。又、任意成分としては、多価アルコール、保湿剤、増粘剤、炭化水素、エステル、アルコール、高級脂肪酸、界面活性剤、粉体成分、色剤、香料、抗酸化剤、紫外線吸収剤、抗炎症剤等が例示できる。更に、含硫化合物やア

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スコルビン酸誘導体、ハイドロキノン誘導体等の他のメラニン生成阻害剤を配合しても構わない。

【0014】皮膚外用剤における、本発明のメラニン生成阻害剤の含有量であるが、0．0001～10重量%が好ましい。これは、含有量が0．0001重量%未満ではメラニン生成阻害活性が期待できず、10重量%を越えても効果が頭打ちで経済的でないためである。皮膚外用剤に於ける本発明のメラニン生成阻害剤の含有量は、メラニン生成阻害作用が更に明瞭である、0．1～10重量%であれば更に好適である。

【0015】

【実施例】以下、実施例を挙げて本発明について更に詳しく説明するが、本発明がこれら実施例に限定を受けない事は言うまでもない。

【0016】実施例1

製造例

瓜科冬瓜の種子である冬瓜子100gに50%メタノール水溶液1lを加え、3時間加熱還流しメラニン生成阻害剤1を淡黄色アモルファスとして7．5g得た。

【0017】実施例2

製造例

上記メラニン生成阻害剤1の7．5gを水300mlに分散させ、300mlのノルマルヘキサンを加え良く振とうした後、分液しノルマルヘキサン層を取り出し、減圧乾固しメラニン生成阻害剤2を淡黄色アモルファスとして170mg得た。残った水層に300mlの酢酸エチルを加え良く振とうした後、酢酸エチル層を取り出し、減圧乾固しメラニン生成阻害剤3を淡黄色アモルファスとして360mg得た。残った水層に300mlのノルマルブタノールを加え良く振とうした後、ノルマルブタノール層を取り出し、減圧乾固しメラニン生成阻害剤4を淡黄色アモルファスとして850mg得た。残った水層を減圧乾固しメラニン生成阻害剤5を淡黄色アモルファスとして5960mg得た。

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【0018】実施例3

メラニン生成阻害作用

実施例1、2のメラニン生成阻害剤1～5について、メラノーマB－16細胞を用いてメラニン生成阻害作用の検討を行った。即ち、対数増殖期にあるB－16細胞をトリプシン処理して、1．5×10³個／ml の濃度の10%FBS（ウシ胎仔血清）含有MEM培地懸濁液とし、これを各培養ボトル10mlづつ分注した。これをCO_2インキュベーター中で2日培養した。（37℃、5%CO_2）これに各種濃度のメラニン生成阻害剤を加え2日間培養を続けた。更にもう一度同じように15ml の培地とメラニン生成阻害剤を加え更に2日間培養した。6日目に培地を除去し、PBS（燐酸緩衝生理食塩水）で洗浄した後トリプシン処理をし細胞をはがした後、遠心分離により細胞を集めこれを肉眼観察し細胞毒性とメラニン生成阻害作用を判定した。細胞毒性の判定基準は、＋：細胞毒性あり、±：細胞毒性の判定が困難、－：細胞毒性無しであった。又、メラニン生成阻害作用の判定基準は＋：メラニン生成阻害作用あり、±：メラニン生成阻害作用がわずかに認められる、－：メラニン生成阻害作用無しであった。結果を表1に示す。本発明のメラニン生成阻害剤は何れも低濃度でもよくメラニンの生成を抑制している事がわかる。

【0019】

【表1】

サンプル	濃度（%）	メラニン生成阻害	細胞毒性
メラニン生成阻害剤1	0.1	＋	＋
メラニン生成阻害剤2	0.004	＋	±
	0.002	＋	－
	0.0002	±	－
メラニン生成阻害剤3	0.01	＋	＋
	0.005	±	－
メラニン生成阻害剤4	0.02	±	－
メラニン生成阻害剤	0.17	±	±

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【0020】実施例4
安全性（局所毒性）
本発明のメラニン生成阻害剤の安全性を知るために、ハートレー系白色種モルモット（雄性、350g）を用いた経皮投与による、局所毒性を検討した。即ち、メラニン生成阻害剤1～5の1%エタノール溶液を、モルモットの背部を剃毛して作成した2cm四方の部位に0．05ml づつ1日1回5日間投与し、6日目に皮膚反応を本邦パッチテスト基準（日本皮膚科学会）に基づいて判*定した。即ち、－：無反応、±：擬陽性反応、＋：陽性反応、＋＋：浮腫を伴う反応である。結果は何れのサンプルも無反応（－）であり、本発明のメラニン生成抑制剤の安全性が高い事がわかる。

【0021】実施例5
配合例（化粧水）
下記の処方に従って化粧水を作成した。即ち、下記成分を秤込み、80℃で加熱溶解し冷却し化粧水を得た。

プロピレングリコール	5．5
エタノール	10
メチルパラベン	0．2
塩化ナトリウム	0．3
クエン酸	0．1
酢酸ナトリウム	0．1
香料	0．1
ポリオキシエチレン（50）硬化ヒマシ油	0．5
水	83．1
メラニン生成阻害剤1	0．1

【0022】実施例6
配合例（化粧水）
※下記の処方に従って化粧水を作成した。即ち、下記成分を秤込み、80℃で加熱溶解し冷却し化粧水を得た。

プロピレングリコール	5．5
エタノール	10
メチルパラベン	0．2
塩化ナトリウム	0．3
クエン酸	0．1
酢酸ナトリウム	0．1
香料	0．1
ポリオキシエチレン（50）硬化ヒマシ油	0．5
水	82．2
メラニン生成阻害剤2	1

【0023】実施例7
配合例（化粧水）
★下記の処方に従って化粧水を作成した。即ち、下記成分を秤込み、80℃で加熱溶解し冷却し化粧水を得た。

プロピレングリコール	5．5
エタノール	10
メチルパラベン	0．2
塩化ナトリウム	0．3
クエン酸	0．1
酢酸ナトリウム	0．1
香料	0．1
ポリオキシエチレン（50）硬化ヒマシ油	0．5
水	73．2
メラニン生成阻害剤1	10

【0024】実施例8
配合例（化粧水）
下記の処方に従って化粧水を作成した。即ち、下記成分を秤込み、80℃で加熱溶解し冷却し化粧水を得た。

プロピレングリコール	5．5
エタノール	10
メチルパラベン	0．2
塩化ナトリウム	0．3
クエン酸	0．1

7	8
酢酸ナトリウム	０．１
香料	０．１
ポリオキシエチレン（５０）硬化ヒマシ油	０．５
水	８３．１９
メラニン生成阻害剤３	０．０１

【００２５】実施例９
配合例（クリーム）
下記の処方に従ってクリームを作成した。即ち、A、B＊をそれぞれ秤込み、８０℃で加熱溶解し、AにBを撹拌しながら徐々に加え乳化した。これを撹拌しながら冷却しクリームを得た。

（A）セタノール	１
合成ゲイロウ	２．５
ミツロウ	２．５
ステアリン酸	１
ワセリン	３
スクワラン	１４
オリーブ油	６
γ－トコフェロール	０．１
香料	０．１
ブチルパラベン	０．１
グリセリルモノステアレート	２．５
ポリオキシエチレン（２５）ステアレート	２．５
（B）苛性カリ	０．０２
メラニン生成阻害剤２	０．０３
水	５６．４
プロピレングリコール	８
メチルパラベン	０．２５

【００２５】実施例１０
配合例（クリーム）
下記の処方に従ってクリームを作成した。即ち、A、B※をそれぞれ秤込み、８０℃で加熱溶解し、AにBを撹拌しながら徐々に加え乳化した。これを撹拌しながら冷却しクリームを得た。

（A）セタノール	１
合成ゲイロウ	２．５
ミツロウ	２．５
ステアリン酸	１
ワセリン	３
スクワラン	１４
オリーブ油	６
γ－トコフェロール	０．１
香料	０．１
ブチルパラベン	０．１
グリセリルモノステアレート	２．５
ポリオキシエチレン（２５）ステアレート	２．５
（B）苛性カリ	０．０２
メラニン生成阻害剤１	０．０００２
水	５６．４２９８
プロピレングリコール	８
メチルパラベン	０．２５

【００２６】実施例１１
配合例（クリーム）
下記の処方に従ってクリームを作成した。即ち、A、Bをそれぞれ秤込み、８０℃で加熱溶解し、AにBを撹拌しながら徐々に加え乳化した。これを撹拌しながら冷却しクリームを得た。

（A）セタノール	１
合成ゲイロウ	２．５

9		10
	ミツロウ	2．5
	ステアリン酸	1
	ワセリン	3
	スクワラン	14
	オリーブ油	6
	γ－トコフェロール	0．1
	香料	0．1
	ブチルパラベン	0．1
	グリセリルモノステアレート	2．5
	ポリオキシエチレン（25）ステアレート	2．5
(B)	苛性カリ	0．02
	メラニン生成阻害剤5	0．3
	水	56．13
	プロピレングリコール	8
	メチルパラベン	0．25

【0027】実施例12
配合例（クリーム）
下記の処方に従ってクリームを作成した。即ち、A、B＊をそれぞれ秤込み、80℃で加熱溶解し、AにBを撹拌しながら徐々に加え乳化した。これを撹拌しながら冷却しクリームを得た。

(A)	セタノール	1
	合成ゲイロウ	2．5
	ミツロウ	2．5
	ステアリン酸	1
	ワセリン	3
	スクワラン	14
	オリーブ油	6
	γ－トコフェロール	0．1
	香料	0．1
	ブチルパラベン	0．1
	グリセリルモノステアレート	2．5
	ポリオキシエチレン（25）ステアレート	2．5
(B)	苛性カリ	0．02
	メラニン生成阻害剤1	0．003
	水	56．427
	プロピレングリコール	8
	メチルパラベン	0．25

【0028】実施例13
配合例（ファンデーション）
下記の処方に従ってファンデーションを作成した。即ち、Aを混練した後Bを加え更に混練し、80℃に加熱した後Cを分散させ、80℃に加熱溶解したDを徐々に加え乳化し、撹拌冷却しファンデーションを得た。

(A)	プロピレングリコール	5
	マルビトール	10
	メチルパラベン	0．3
	ジグリセリントリイソステアレート	4
(B)	流動パラフィン	5
	ブチルパラベン	0．1
(C)	酸化チタン	9
	黄色酸化鉄	1．7
	ベンガラ	1．2
	タルク	8．1
(D)	水	55
	メラニン生成阻害剤5	0．6

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【0029】

【発明の効果】本発明のメラニン生成阻害剤は、安全性が高く、且つ、メラニン生成阻害作用も高いので大変有益である。従って、これを含有する皮膚外用剤も安全性が高く、優れたメラニン生成阻害作用を有するのでたいへん有益である。

フロントページの続き

(72)発明者　原　烈
神奈川県横浜市神奈川区高島台27番地1
ポーラ化成工業株式会社横浜研究所内

(72)発明者　大畑　智
神奈川県横浜市神奈川区高島台27番地1
ポーラ化成工業株式会社横浜研究所内

(72)発明者　中島　琢自
神奈川県横浜市神奈川区高島台27番地1
ポーラ化成工業株式会社横浜研究所内

(72)発明者　宮田　善之
神奈川県横浜市神奈川区高島台27番地1
ポーラ化成工業株式会社横浜研究所内

(72)発明者　北田　好男
神奈川県横浜市神奈川区高島台27番地1
ポーラ化成工業株式会社横浜研究所内